IUCLID

Data Set

Existing Chemical	:	ID: 50594-77-9
CAS No.	:	50594-77-9
Producer related part		
Company	:	The Dow Chemical Company
Creation date	:	25.04.2003
Substance related part		
Company	:	The Dow Chemical Company
Creation date	:	25.04.2003
Status	:	
Memo	:	
Printing date	:	01.09.2005
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Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	
Name	:	The Dow Chemical Company
Contact person	:	
Date	:	
Street	:	2020 Dow Center
Town	:	48674 Midland, Michigan
Country	:	United States
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	

25.04.2003

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	
Substance type	:	organic
Physical status	:	liquid
Purity	:	= 47.5 - 51.3 % w/w
Colour	:	
Odour	:	

Remark :

Common Name : RH-35,201 Crude
Chemical Name : 2-chloro-4-trifluoromethyl -3'-acetoxydiphenyl ether
CAS N0 : 50594-77-9
Purity/Potency/Assay: RH-35,201 - 51.33% (by GLP analysis)
Purity/Potency/Assay after Re-analys: RH-35,201 - 47.5% (by GLP analysis)
Batch N0/Lot N0 : Lot # 1222
Date of Manufacture : September 24, 1999
Manufactured by : Rohm and Haas Company
Physical Appearance : Yellow liquid

06.05.2003

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

2-chloro-4-trifluoromethyl-3'-acetoxydiphenyl ether

25.04.2003

RH-35,201 Crude

Source : Dow Agrosciences, LTD.
Dow Agrosciences, LTD.

25.04.2003

1.3 IMPURITIES

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value	:	= -16 °C
Sublimation	:	
Method	:	OECD Guide-line 102 "Melting Point/Melting Range"
Year	:	2002
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The melting point/ freezing point corresponds to the temperature at which the phase transition; solidification (for liquid compound) at normal atmospheric pressure takes place. Method to Determine the Freezing Point The test substance was filled in a test tube and placed in a cryostat water bath apparatus (Ultra Low Cryostat Bath/Circulator" UCB-30) for the determination of the freezing point. The instrument is designed to operate from + 400C (using heater) to -300C (using refrigeration). The sample was stirred gently and continuously during cooling and the temperature was recorded at 30 seconds intervals starting from 00C to -240C with a calibrated thermometer. The temperature remained constant at -160C for 10 readings (for 300 seconds), this temperature at which phase transition had taken place, was considered as the crystalline point or freezing point of the test substance. The experiment was conducted only once.
Result	:	The RH-35,201 crude was tested for its melting temperature (freezing temperature) and it was observed that the melting point of RH-35,201 crude was -160C.
Source	:	Dow Agrosciences, LTD
Reliability	:	(1) valid without restriction

06.05.2003 (1)

2.2 BOILING POINT

Value	:	= 185.3 °C at
Decomposition	:	
Method	:	OECD Guide-line 103 "Boiling Point/boiling Range"
Year	:	2002
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	A few ml volume of the RH-35,201 Crude was heated in the sample tube (5 mm diameter) in the heating bath of the melting point apparatus [Veego TID 2000 MP-D]. A capillary tube fused about 1 cm above the lower end was placed in the sample tube. The level to which the test substance added was such that the fused section of the capillary is below the surface of the liquid. The sample tube was placed in the liquid paraffin bath of the melting point apparatus. The heating of the bath was adjusted to a temperature rise of 30C per minute. Near the boiling temperature, bubbles were beginning to emerge from the capillary tube. At about 100C below the boiling temperature, the heating was reduced so that the rate of temperature rise was 10C/minute to obtain an accurate boiling temperature. Near the boiling temperature when a string of bubbles began to emerge from the capillary tube, the heating was stopped and on momentary cooling the fluid would suddenly start rising in the capillary. The corresponding temperature reading was the boiling temperature of the RH-35,201 Crude. The experiment was repeated thrice and the mean value

was reported.

Remark : Siwoloboff Method was used.
Result : The RH-35, 201 Crude was tested for its boiling temperature and it was observed that the mean boiling point of RH-35,201 Crude was 185.3 1 1.150C.
Source : Dow Agrosciences, LTD.
Reliability : (1) valid without restriction
06.05.2003 (1)

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

2.5 PARTITION COEFFICIENT

Partition coefficient :
Log pow : at 25 °C
pH value :
Method : OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year : 2002
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : Method Validation

Linearity

Preparation of Standard Solutions for Linearity

A quantity of 53.0 mg of the reference standard of RH-35,201 (94.52 %) was weighed into a volumetric flask of 50 ml capacity, dissolved in 10 ml of acetonitrile (HPLC grade) and the volume was made up to the mark with acetonitrile (stock solution of 1000 ppm). From the above stock solution, standard solutions of 100, 50, 25, 10, 5 and 1 ppm were prepared by serial dilution. The standard solutions prepared were injected onto the HPLC using validated method. The linear calibration curve was prepared by plotting the mean peak areas against concentration.

Precision (% RSD)

The precision of analytical method was done by injecting 10 replicate samples of RH-35,201 CRUDE and assayed for RH-35,201 active ingredient content in each replicate. Preparation of Standard Solution for Precision Determination A quantity of 5.3 mg of the reference standard of RH-35,201 (94.52 %) was weighed into a volumetric flask of 10 ml capacity, dissolved in 5 ml of acetonitrile (HPLC grade) and the volume was made up to the mark with acetonitrile.

Preparation of Sample Solutions for Precision Determination

A quantity of 5.3, 6.3, 7.4, 8.4, 9.0, 10.6, 11.4, 12.1, 12.9 and 14.5 mg of RH-35,201 CRUDE samples were weighed into separate volumetric flasks

of 10 ml capacity, 5 ml of acetonitrile (HPLC grade) was added to dissolve the material and the volume was made up to the mark with acetonitrile.

Accuracy (% Recovery)

Accuracy of analytical method was determined by injecting three sample of RH-35,201 CRUDE fortified with reference standard.

Preparation of Standard Solution for Accuracy Determination

The reference standard solution prepared for precision was used for accuracy determination.

Preparation of Sample Solutions for Accuracy Determination

A quantity of 10.2, 10.1 and 10.3 mg of RH-35,201 CRUDE samples were weighed into separate volumetric flasks of 10 ml capacity and fortified with 9.82, 9.92 and 9.73% (w/w) RH-35,201 standard (purity 94.52%), respectively and the volume was made up to the mark with acetonitrile.

HPLC Parameters

The above prepared standard solutions and sample solutions of RH-35,201 curve were injected on to the High Performance Liquid Chromatograph (HPLC) using following parameters :

Instrument : HPLC (Shimadzu LC - 10 AT pump, SPD-10A detector with LC-10 software)
Column : ODS [25 cm X 4.6 mm (i.d.), 5 5m particle size]
Column manufacturer : SGE Laboratory Accessories Pvt. Ltd.
Wave length : 213 nm.
Mobile Phase : Acetonitrile : Water (60:40) Flow Rate : 2.0 ml / min.
Injection Volume : 20 ml
Range : 0.036 AUFS

Calculation

RH-35,201 active ingredient content present in sample was identified and quantified by injecting the reference standard of known purity. The external standard quantitation based on the peak area of the reference standard to the peak area of the sample was used.

Presaturation of Solvents

Before determining the partition coefficient the two solvents (n-octanol and water) were mutually saturated by shaking at the room temperature for 24 h. A 250 ml volume of both the solvents were taken in a bottle and shaken mechanically by using end-over-end shaker for 24 hours. The solvents were then allowed to stand for 30 minutes for clear separation of the phases. The two solvents were then separated and used for partition coefficient determination.

Preparation of Test Substance Stock Solution

A quantity of 24.4 mg of RH-35,201 CRUDE was weighed in a volumetric flask of 100 ml capacity. The test substance was dissolved in 20 ml of n-octanol (pre-saturated) and the volume was made up to the mark with n-octanol.

Preparation of Reference Standard Solution

A quantity of 26.4 mg of reference standard of RH-35,201 was weighed in a volumetric flask of 25 ml capacity, dissolved in 10 ml of acetonitrile (HPLC grade) and the volume was made up to the mark with acetonitrile. The resulting solution had concentration of 1000 ppm. From this solution, four other lower concentrations viz., 25, 10, 5 and 1 ppm were prepared by serial dilutions.

Test Substance Application and Separation

The two solvents (n-octanol and water) were added in the ratio of 24:25 ml, 24: 50 ml and 49:25 ml in 250 ml separatory funnels in duplicate. To all the six systems 1.0 ml of RH-35,201 CRUDE stock solution was added (prepared in n-octanol) to get a final n-octanol and water ratio of 1:1, 1:2 and 2:1. The test vessels were shaken vigorously by hand and the contents were allowed to stand for 3 hours. The aqueous and organic layers were separated and analysed separately for the RH-35,201 CRUDE active ingredient content by HPLC using validated parameters.

Preparation of Calibration Curves and Calculation of Active Ingredient

The standard solutions of 1, 5, 10 and 25 ppm were injected onto the HPLC using validated method. The linear calibration curves were prepared by plotting the peak areas against concentration of RH-35,201 standard solutions. The linear calibration curve was prepared for day I and II, separately. From the constants intercept (a), slope (b) Y on X and regression coefficient (r) were derived.

Result : Method Validation

Validation of the method was done before initiating the analysis of RH-35,201 CRUDE sample. The method validation covers the aspect of linear dynamic range, precision and accuracy.

Linear Dynamic Range

The linear dynamic range to establish the linear calibration curve was arrived by injecting six different concentrations of RH-35,201 standard in duplicate and by plotting the mean peak areas against the concentration.

Precision (% RSD)

Precision (% RSD) of analytical method for RH-35,201 was determined by analysing 10 replicate samples of RH-35,201 CRUDE and assayed to quantify the active ingredient content in each replicate. The precision was 0.50%.

Accuracy (% Recovery)

Accuracy (% Recovery) of analytical method for RH-35,201 was determined by fortifying the RH-35,201 CRUDE sample with reference standard in three replication. The mean accuracy was 98.55%.

Calibration

The linear calibration curve was arrived by injecting four different concentrations of RH-35,201 standard and by plotting the peak areas against the concentration.

Partition Coefficient

The active ingredient content in n-octanol phase determined in the ratio of 1 : 1, 1 : 2 and 2 : 1 was 6.02, 4.90 and 2.38 ppm, respectively. The active ingredient content of RH-35,201 detected in n-octanol phase was

equivalent to the quantity of active ingredient content added in various ratios viz., 1 : 1, 1 : 2 and 2 : 1. The active ingredient content in water phase in all ratios (1 : 1, 1 : 2 and 2 : 1) was non-detectable. Since RH-35,201 active ingredient content was not detected in water phase, the calculation of partition coefficient was not practicable.

Source : The Dow Chemical Company, Midland, MI
Conclusion : The n-octanol and water phases from difference ratios (1:1, 1:2, and 2:1) were analyzed for the material. As the material was not detected in the water phase, the calculation of partition coefficient was not practicable.
Reliability : (1) valid without restriction
06.05.2003 (2)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in :
Value : = .54 g/l at 25 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : OECD Guide-line 105
Year : 2002
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : Preliminary Test

A quantity of 0.1073 g of test substance was weighed and a preliminary estimation of solubility was made by adding increasing volumes of water to the container with test substance. As the test substance was not soluble up to 1000 ml of distilled water, the estimated solubility was < 0.1 g/l.

Column Elution Method

A quantity of 0.1028 and 0.1030 g of RH-35,201 CRUDE was weighed into two separate beakers, the test substance was dissolved in 1 ml of dichloromethane and transferred to round bottom flask. A quantity of 100.9 and 100.3 mg of silica gel was added to the respective flasks. The slurry thus obtained was dried under vacuum. The dried slurry was then loaded in the two micro columns (length 150 mm, 5 mm i.d.) No 1 & 2, having 600.9 and 600.5 mg of silica gel as a carrier material, respectively. After loading the test substance mixed with silica gel, columns No 1 and 2 were eluted with distilled water at the flow rate of 25 ml/h and 12.5 ml/h, respectively (approximately 10 bed volume). First five bed volumes were discarded to remove the impurities of water or samples. Successively 8 fractions were collected. The collected fractions were injected directly onto HPLC for quantitation. The pH and temperature of all fractions were also recorded. The last five consecutive sample fractions from the saturation plateau were considered for water solubility calculation and the last three consecutive sample fractions from the saturation plateau were considered for water solubility calculation at the flow rate of 25 ml/h and 12.5 ml/h.

Methodology of Analysis

Preparation of RH-35,201 Reference Standard Solutions
A quantity of 26.4 mg of the reference standard of RH-35,201 (purity

94.52%) was weighed in to a volumetric flask of 25 ml capacity, dissolved in 5 ml of acetonitrile (HPLC grade) and the volume was made up to the mark with acetonitrile to obtained 1000 ppm solution. From the above prepared solution further serial dilutions were made to prepare 100, 50, 25, 10 and 1 ppm solutions.

Linear Dynamic Range

Linear dynamic range was established by injecting 100, 50, 25, 10 and 1 ppm standard solutions on to the HPLC and by plotting the peak area for each solution (Refer Table 1 & Figure 1) against concentration.

Chromatographic Conditions
Instrument : HPLC (Shimadzu LC - 10 AT pump, SPD-10A detector with LC-10 software)
Column : ODS [25 cm X 4.6 mm (i.d.), 55m particle size]
Column manufacturer: SGE Laboratory Accessories Pvt. Ltd.
Wavelength : 213 nm.
Mobile Phase : Acetonitrile : Water (60:40)
Flow Rate : 2.0 ml / min.
Injection Volume: 20 ml
Range : 0.036 AUFS

Calculation

The active ingredient content of RH-35,201 CRUDE in each fraction was calculated using the following formula :

$$\text{A. I. Content (ppm) } X = \frac{Y - a}{b}$$

where

X = A.I. content (ppm) @ 5g/ml @ mg/l
Y = Area of sample
a = Constant
b = Regression co-efficient for Y on X

Result : The solubility of active ingredient of RH - 35,201 CRUDE in water at the flow rate of 25.0 ml/h was 0.54 +/- 0.022 x 10-3 g/l and at the flow rate of 12.5 ml/h was 0.53 +/- 0.006 x 10-3 g/l by column elution method. The mean solubility at both flow rate was 0.54 +/- 0.02 x 10-3 g/l.

Source : Dow Agrosciences, LTD.

Conclusion : The solubility of active ingredient of RH - 35,201 CRUDE in water at the flow rate of 25.0 ml/h was 0.54 1 0.022 x 10-3 g/l and at the flow rate of 12.5 ml/h was 0.53 1 0.006 x 10-3 g/l by column elution method. The mean solubility at both flow rate was 0.54 1 0.02 x 10-3 g/l.

Reliability : (1) valid without restriction
28.07.2003 (1)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	= .0000000000041751 cm³/(molecule*sec)
Degradation	:	= 50 % after 2.6 day(s)
Deg. product	:	
Method	:	other (calculated)
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method : Photodegradation was estimated using models accepted by the EPA 1. An estimation method accepted by the EPA includes the calculation of atmospheric oxidation potential (AOP). Atmospheric oxidation as a result of hydroxyl radical attack is not direct photochemical degradation, but rather indirect degradation. AOPs can be calculated using a computer model.

The computer program AOPWIN (atmospheric oxidation program for Microsoft Windows) 2 is used by The Dow Chemical Company. This program calculates a chemical half-life based on an overall OH reaction rate constant, a 12-hr day, and a given OH concentration.

Source : The Dow Chemical Company, Midland, MI.
Reliability : (1) valid without restriction
31.08.2005

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	= 29.9 day(s) at 25 °C
t1/2 pH9	:	at °C
t1/2 pH 8	:	= 3 day(s) at 25 °C
Deg. product	:	
Method	:	other (calculated)
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method : Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters 3. Stability in water can be estimated using models accepted by the EPA 1. An estimation method accepted by the EPA includes a model that can calculate hydrolysis rate constants for esters, carbamates, epoxides, halomethanes, and selected alkylhalides. The computer program HYDROWIN (aqueous hydrolysis rate program for Microsoft windows) 2 was used for hydrolysis calculation.

Source : The Dow Chemical Company, Midland, MI.
Reliability : (1) valid without restriction
22.05.2003

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	other
Concentration	:	2 mg/l related to Test substance related to
Contact time	:	28 day(s)
Degradation	:	ca. 40 (±) % after 28 day(s)
Result	:	other
Kinetic of testsubst.	:	7 day(s) = 24.7 % 14 day(s) = 31.2 % 21 day(s) = 37.6 % 28 day(s) = 39.78 % %
Control substance	:	other
Kinetic	:	7 69.8 % 28 78.3 %
Deg. product	:	not measured
Method	:	OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year	:	2002
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Preparation of Test Solution

Stock solution of Test substance

The stock solution of RH-35,201 Crude was prepared by dissolving 200 mg of RH-35,201 Crude in 10 ml of dimethyl sulfoxide (DMSO) and diluted up to 100 ml with double distilled water. This solution contains 2 mg RH-35,201 Crude and 0.1 ml DMSO per ml.

Test solution of Test substance

The test solution of test substance was prepared by mixing 4 ml of stock solution of RH-35,201 Crude in 3994.4 ml of mineral medium (2 mg/l) in a 5 liter conical flask. A quantity of 1.6 ml of inoculum was added to the mineral medium and mixed thoroughly.

Stock and Test Solution of Vehicle Control

The stock solution of vehicle control was prepared by diluting 5 ml of dimethyl sulfoxide (DMSO) up to 50 ml with double distilled water. This solution contains 0.1 ml / ml DMSO.

The test solution of vehicle control was prepared by mixing 4 ml of stock solution of vehicle in 3994.4 ml of mineral medium (0.1 ml/l) in a 5-liter conical flask. A quantity of 1.6 ml of inoculum was added to the mineral medium and mixed thoroughly.

Test Solution of Reference Substance

The test solution of reference substance was prepared by dissolving 8 mg of potassium hydrogen phthalate in 3998.4 ml of mineral medium (2 mg/l). A volume of 1.6 ml of inoculum was added to the mineral medium and mixed properly.

Test Solution of Control

The stock solution for control was prepared by adding 1.6 ml of inoculum to 3998.4 ml of mineral medium.

Procedure

The solution of test substance, reference substance, vehicle control and control was dispensed to respective pre-labeled BOD bottles in duplicate for the analysis of dissolved oxygen on day 0, 7, 14, 21 and 28. Each series of test substance and reference substance solution were accompanied by a parallel series of control. The zero day samples were analysed for dissolved oxygen immediately after dispensing.

Dissolved oxygen (DO) was determined using a dissolved oxygen meter. The samples for analysis of DO on day 7th, 14th, 21st and 28th were maintained at 20 1 2 :C in a BOD incubator.

Chemical Oxygen Demand (COD)

The chemical oxygen demand of RH-35,201 Crude was determined by open reflux method. For this purpose 10 mg of RH-35,201 Crude was taken in round bottom flask and suspended in 50 ml double distilled water. One gram HgSO4 and several glass beds were added to this followed by 5 ml of sulfuric acid reagent (5.5 g AgSO4 / Kg H2SO4) and mixed to dissolve HgSO4. The flask was cooled to reduce the loss of volatile organic matter. Then 50 ml of 0.25N K2Cr2O7 solution was added to the mixture and mixed properly. Then the content was refluxed for 2 hours. The content was cooled to room temperature and diluted with double distilled water. The mixture was titrated with standard (previously standardized with 0.25N K2Cr2O7 solution) ferrous ammonium sulfate solution (FAS) using 3 drops of ferroin indicator. The end point of the titration was sharp colour change from blue green to reddish brown. One blank was run in the similar way without taking any sample.

Remark : Potassium hydrogen phthalate was used as a control.
River water collected from Daman Ganga River, Vapi, Gujarat, India was used as inoculum.

Result : Dissolved Oxygen in Test Solution

The mean values of dissolved oxygen in control on 0, 7th, 14th, 21st and 28th day were 8.99, 8.36, 8.06, 7.86 and 7.79 mg/l respectively; in vehicle control on 0, 7th, 14th, 21st and 28th day were 8.97, 8.31, 7.95, 7.77 and 7.67 mg/l respectively; in RH-35,201 Crude were 9.06, 7.94, 7.46, 7.16 and

7.02 mg/l respectively, and in potassium hydrogen phthalate were 9.03, 6.76, 6.41, 6.09 and 6.00 mg/l respectively.

Oxygen Consumption

Oxygen consumption due to reference substance (compare with control) was 1.64, 1.69, 1.81, 1.83 mg/l and due to test substance (Compare with vehicle control) was 0.46, 0.58, 0.70, 0.74 mg/l on 7th, 14th, 21st and 28th day, respectively.

BOD and Per cent Degradation

The Biological Oxygen Demand (BOD) values for test substance on 7th, 14th, 21st and 28th day were 0.23, 0.29, 0.35 and 0.37 mg O2/mg test substance. The Chemical Oxygen Demand (COD) for RH-35,201 Crude was 0.93 mg/mg and the per cent degradation on 7th, 14th, 21st and 28th were 24.73, 31.18, 37.63 and 39.78, respectively.

The Biological Oxygen Demand (BOD) values for reference substance on 7th, 14th, 21st and 28th day were 0.82, 0.85, 0.91 and 0.92 mg O2/mg reference substance. The Theoretical Oxygen Demand (ThOD) for potassium hydrogen phthalate was 1.175 mg/mg and the per cent degradation on 7th, 14th, 21st and 28th were 69.79, 72.34, 77.45 and 78.30, respectively. The biodegradation of reference substance was more than 60 % (based on ThOD) at the end of study period. Hence, the reference substance (potassium hydrogen phthalate) was ready biodegradable.

Source : The Dow Chemical Company, Midland, MI.

Conclusion : The biodegradability of RH-35,201 Crude was 24.73, 31.18, 37.63 and 39.78% on 7th, 14th, 21st and 28th days, respectively. The per cent biodegradation data revealed that the substance is not ready biodegradable. Where as the reference substance exhibited a normal pattern of degradation (up to 78.30%) within 28 days. Therefore it can be concluded that RH-35,201 Crude is not readily biodegradable.

During the experiment the total dissolved oxygen consumption in control was 1.20 mg/l after 28 days (guideline limits = 1.5 mg/l, for inoculum blank) and the residual concentrations of oxygen in the test bottles on day 28 was 7.02 mg/l (guideline limits > 0.5 mg/l at any time).

Reliability : (1) valid without restriction
06.05.2003 (2)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	semistatic
Species	:	Oncorhynchus mykiss (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= .1 measured/nominal
LC0	:	= .1 measured/nominal
LC50	:	= .32 calculated
LC100	:	= .84 measured/nominal
Limit test	:	
Analytical monitoring	:	yes
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	2002
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Main Study

A preliminary study was conducted with test concentrations of 0.1, 1.0, 10.0 and 100.0 mg RH-35,201 Crude/l water for a period of 96 h with ten fish in each group along with control and vehicle control group to determine the dose levels for the main study. The per cent mortalities observed were 0, 100, 100 and 100% at the test concentrations of 0.1, 1.0, 10.0 and 100 mg RH-35,201 Crude/l. No mortalities were observed in the control and vehicle control groups.

A quantity of 6.5, 11.05, 18.85, 31.85 and 54.6 mg of RH-35,201 Crude was weighed for each test concentration and was dissolved in acetone and further dispersed in 65 litres of water taken in the glass aquarium of 80 litres capacity to obtain the desired concentration in the test tank (aquarium) of each group. The test solutions were thoroughly mixed to achieve a uniform distribution of the test substance. The tank water was changed every 24 h with freshly prepared (15 minutes prior to exposure) test substance solution. The test concentrations of control (without RH-35,201 Crude), vehicle control (0.05 ml acetone/l), 0.1, 0.17, 0.29, 0.49 and 0.84 mg RH-35,201 Crude/l were used. The photoperiod of 16 h light and 8 h dark was maintained, with the help of an automatic timer attached to the power supply.

Analysis of Test Concentration
A volume of 100 ml of water sample was taken in a separatory funnel, added 50 ml of ethyl acetate, shaken well and the separated oraganic layer was collected. The above process was repeated again with 50 ml of ethyl acetate and the organic layer was collected. The combined organic layer was concentrated in rotary vacuum evaporator at 450C. The residue obtained was dissolved in 5 ml of ethyl acetate (A.R. grade) and analysed in GC. The a.i. content was determined from the standard and sample peak area responses using the following formula.

$$\text{A.I. Content (ppm) } X = \frac{Y - a}{b} \times \frac{5}{100}$$

Y = Peak area of the sample
a = Constant
b = Regression Co-efficient for Y on X

A.I. content
Test Substance Concentration = ----------
0.5133

Observations

Groups of 10 fish per dose level were observed at 3, 6, 24, 48, 72 and 96 h of exposure for behavioural responses, clinical signs and mortality.

Water Quality Parameters

Temperature, pH and dissolved oxygen content of the test media were measured daily before and after each change of media with freshly prepared test solution. Total hardness was analysed daily for the diluent water during the study period.

Statistical Analysis of Results
From the mortality data an estimate of 96 h LC50 of RH-35,201 Crude was made together with 95% confidence limits using the Probit analysis method (Finney, 1971).

Result : Mortalities

The per cent mortalities (96 h) observed were 0, 10, 40, 70 and 100 at the test concentrations of 0.1, 0.17, 0.29, 0.49 and 0.84 mg RH-35,201 Crude/l, respectively. No mortalities were recorded in the control (without RH-35,201 Crude) and vehicle control (0.05 ml acetone/l water) groups.

Observations

The clinical symptoms observed were loss of equilibrium, dark pigmentation, wide opened operculum and opened mouth at the test concentration of 0.84 mg RH-35,201 Crude/l. Dark pigmentation, swimming on surface, wide opened operculum and opened mouth were observed at the test concentration of 0.49 mg RH-35,201 Crude/l. Dark pigmentation, swimming on surface and opened mouth were observed at the test concentrations of 0.29mg/l were observed. Dark pigmentation and opened mouth were observed at the test concentrations of 0.17mg/l were observed. No clinical symptoms were observed at the concentration of 0.1 mg/l, control and vehicle control groups.

Water Quality Parameters

RH-35,201 Crude did not alter the selected water quality parameters.

Test Level	pH		Temperature (degrees C)		Dissolved Oxygen (%)	
	I	F	I	F	I	F
Control	7.6	7.8	15.9	16.5	87.7	75.4
Vehicle	7.6	7.8	16.1	16.6	88.7	75.8
0.1	7.7	7.9	16.2	16.6	88.3	70.0
0.17	7.7	7.9	16.1	16.6	88.8	70.0
0.29	7.7	7.9	16.2	16.6	89.8	68.6
0.49	7.7	7.8	16.1	16.6	90.4	68.4
0.84	7.7	7.9	16.2	16.6	91.1	68.9

I = Inital
F = Final

Total Water Hardness (as CaCO3) for all test levels: 214.0 mg/l.

Analysis of Test Concentration

Based on the purity of the test substance the active ingredient content of the test solution were analytically determined at 0 and 24 h. The analytical concentrations remained > 90% of the active ingredient content.

Statistical Analysis

The 96 h acute median lethal (LC50) of fish due to the exposure of RH-35,201 Crude was determined by using the analytical concentrations obtained and probit of mortality using the Probit analysis method (Finney's, 1971).

The 96 h LC50 of RH-35,201 Crude was determined as 0.32 mg/l. The confidence limits to LC50 were found to be between 0.23 and 0.45 mg/l. The regression equation established [Probit mortality (Y) vs log concentration (mg/l) of RH-35,201 Crude (X)] was, Y= 6.787 + 3.627 X.

Source : The Dow Chemical Company, Midland, MI.

Conclusion : Based upon the mortality data, the 96 h LC50 of RH-35,201 Crude was determined as 0.32 mg/l. The confidence limits to LC50 were found to be between 0.23 and 0.45 mg/l. The regression equation established [Probit mortality (Y) vs log concentration (mg/l) of RH-35,201 Crude (X)] was, Y= 6.787 + 3.627 X.

Reliability : (1) valid without restriction

01.09.2005 (2)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	= 3 measured/nominal
EC0	:	= 3 measured/nominal
EC50	:	= 4.9 measured/nominal
EC100	:	= 11.5 measured/nominal
Analytical monitoring	:	yes
Method	:	OECD Guide-line 202
Year	:	2002
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Test Groups

Daphnids (ca. 24 h old) numbering 140 were divided into seven equal groups and each group with four replicates, each replicate comprised 5 daphnids.

Preparation of Test Substance Concentrations

An amount of 4.8, 6.72, 9.44, 13.12 and 18.4 mg RH-35,201 Crude were weighed and each were dissolved separately in 1 ml acetone and made upto 20 ml in distilled water. These stock solutions were used for the replicates of each group. Stock solutions were prepared freshly 15 minutes prior to the exposure. Test solutions were not changed during the 48 h exposure period. Based on the preliminary range finding study, the test concentrations selected for the main study were 3.0, 4.2, 5.9, 8.2 and 11.5 mg RH-35,201 Crude/l for test groups. Another group of 40 daphnids, with 20 daphnids per group and 5 daphnids per replicate were exposed only to water [without RH-35,201 Crude] and with vehicle only (acetone 0.1 ml/l). These groups served as the control and vehicle control groups. Test

containers along with the daphnids were maintained in experimental room at 16 h light and 8 h darkness.

Daphnid Selection

First instar daphnids (# 24 h old), were separated from the adults in the culture of daphnia being acclimatised for the study and were immediately transferred to labelled glass beakers (one for each concentration). The separation was performed using a micropipette having a capacity of 100 - 1000 5l along with a known volume of culture water. The mobility of the test organisms were verified immediately prior to the commencement of exposure by gentle swirling of the container with visual inspection.

Water samples were collected from the test beakers of each group, after thoroughly mixing the test solution of 0 h and 48 h for the analysis of active ingredient content and stability.

Analysis of Test Concentrations

100 ml of the water sample was taken in a separatory funnel, added 50 ml of ethyl acetate, shaken well and the separated organic layer was collected. The above process was repeated again with 50 ml of ethyl acetate and the organic layer was collected. The combined organic layer was concentrated in rotary vacuum evaporator at 45 0C. The residue obtained was dissolved in 5 ml ethyl acetate (A.R. grade) and analysed in GC. The a.i. content was determined from the standard and sample peak area responses using the following formula:

$$\text{A.I. Content (ppm) } X = \frac{Y - a}{b} \times \frac{5}{100}$$

Y = Peak area of the sample
a = Constant
b = Regression Co-efficient for Y on X

$$\text{Test Substance Concentration} = \frac{\text{A.I. content}}{0.5133}$$

Observations

All test daphnids were observed for mobility at 24 and 48 h after the commencement of exposure to the respective test concentration. Mobility was assessed by gentle tapping of test container for 15 seconds and observing the movement of the test organisms. Those daphnids unable to swim during the 15 seconds test period and organisms remaining settled on the water surface even after agitation of containers, were recorded as immobilised.

Temperature, pH and dissolved oxygen content and of test media were measured at 0 and 48 h after the commencement of exposure. Total hardness of the test media was measured during the study period.

Statistical Analysis of Results

From the immobility data, an estimate of the acute (24 and 48 h) immobilisation concentration (EC50) of RH-35,201 Crude were made together with the 95% confidence limits using the Probit analysis method (Finney, 1971).

Result : Immobility

The per cent immobilisation observed at 24 h were 0, 20, 40, 55 and 60

at the test concentrations of 3.0, 4.2, 5.9, 8.2 and 11.5 mg RH-35,201 Crude/l, respectively. There were no immobilisations recorded in the control and vehicle control groups at 24 h.

The per cent immobilisation observed at 48 h were 0, 35, 60, 70 and 100 at the test concentrations of 3.0, 4.2, 5.9, 8.2 and 11.5 mg RH-35,201 Crude/l, respectively. There were no immobilisations recorded in the control and vehicle control groups 48 h.

Water Quality Parameters

RH-35,201 Crude did not alter the selected water quality parameters.

Test Level	pH at		Temperature (degrees C) at		Dissolved Oxygen (%) at	
	0h	48h	0h	48h	0h	48h
Control	7.56	7.75	20.9	21.6	68.1	66.7
Vehicle	7.57	7.81	21.0	21.8	68.1	67.6
3.0	7.57	7.82	20.9	21.8	68.5	67.8
4.2	7.57	7.80	20.9	21.8	68.4	67.6
5.9	7.56	7.80	21.1	21.8	68.0	67.2
8.2	7.57	7.81	20.9	21.8	68.4	67.5
11.5	7.57	7.83	20.3	21.8	68.5	67.4

Analysis of Test Concentrations

Based on the purity of the test substance the active ingredient content of the test solutions were analytically determined at 0 and 48 h. The analytical concentration remained >90% of the nominal concentration.

Statistical Analysis

The 48 h acute immobilization (EC50) of daphnia due to the exposure of RH-35,201 were determined by taking the average of analytical concentrations obtained at 0 and 48 h, instead of the nominal concentrations and probit of immobility using the probit method of analysis (Finney, 1971).

The 24 h EC50 value is 7.6 mg/l with 5.7 and 10.2 mg/l as lower and upper confidence limits. The 48 h EC50 value is 4.9 mg/l with 3.8 and 6.4 mg/l as lower and upper confidence limits.

Source : The Dow Chemical Company, Midland, MI.

Conclusion : Based on the average of analytical concentration at 0 and 48 h, the 24 and 48 h EC50 of RH-35,201 Crude in Daphnia magna were calculated.

The 24 h EC50 value is 7.6 mg/l with 5.7 and 10.2 mg/l as lower and upper confidence limits. The regression equation Y = 2.880 + 2.407 X.

The 48 h EC50 value is 4.9 mg/l with 3.8 and 6.4 mg/l as lower and upper confidence limits. The regression equation Y = 2.854 + 3.101 X.

Reliability : (1) valid without restriction

31.08.2005 (1)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)

Endpoint : biomass

Exposure period : 96 hour(s)

Unit : mg/l

NOEC : = .03 measured/nominal

EC50	:	= .131 calculated
Limit test	:	
Analytical monitoring	:	no
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	2002
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Main Study

Based on the results of preliminary dose range finding study, the test concentrations of RH-35,201 Crude selected were 0.03, 0.06, 0.12, 0.24 and 0.48 mg/ml (nominal). Three replicates were maintained for each concentration whereas six replicates were maintained for the control groups.

Test Concentration Preparation

The cell concentration of the algal stock culture was determined as 1177500 cells/ml, using a haemocytometer. In each replicate one ml of algal culture was added to obtain the required cell concentration (approximately 1 x 104). One ml of the relevant stock solution (solutions E to I) was added to each of the three replicates to obtain the nominal test concentrations of 0.48, 0.24, 0.12, 0.06 and 0.03 mg RH-35,201 Crude/ml. Final volume was made up with sterilised culture medium, to 100 ml (using measuring cylinder) and transferred to 250 ml labelled conical flask. The control was maintained with six replicates but devoid of the test substance. One ml of the relevant stock solution (Stock V) was added to six replicates of vehicle control but devoid of the test substance. The vehicle control (acetone) was maintained with the highest concentration used in the test solution. The initial mean cell concentration in the test medium was calculated as 11775 cells/ml.

Test Culture Maintenance

The cells in the culture flasks were maintained in suspension by agitating the test flasks continuously, using an orbital shaker (Orbitek -LX-IL) during the study period. The culture flasks were maintained at 23 1 20C for 96 h. Illumination and temperature were recorded daily using a light meter (Lutron, LX-102) and minimum maximum thermometer respectively. The pH of both the control groups and test concentration cultures were measured at 0h and 96h using a pH analyser (ORLAB, OR 103)

Algal Cell Counts
A quantity of 10 ml of the test culture was collected from each replicate flask at 24, 48, 72 and 96 h and the cell concentration of each sample was determined using a haemocytometer and microscope (Labomed-Vision-2000)

The percentage inhibition of the cell growth at each test substance concentration (IA) was calculated as the difference between the area under the control growth curve (Ac) and the area under the growth curve at each test substance concentration (At) as :

$$IA = \frac{Ac - At}{Ac} \times 100$$

The EC50 derived by this method was specified as EbC 50.

Calculation of Growth Rate
The average specific growth rate (m) for exponentially growing cultures

was calculated using the formula

$$m = \frac{\ln Nn - \ln N1}{tn - t1}$$

The percentage reduction in average growth rate (Imt) at each test substance concentration compared to the control value was calculated using the following formula:

$$Imt = \frac{mc - mt}{mc} \times 100$$

mc = mean control specific growth rate
mt = mean specific growth rate for the test concentration t

The EC50 derived by this method was specified as ErC 50.

EC50 (0-96h) was calculated using the Probit analysis (Finney, 1971) method through validated computer software.

Calculation of NOEC
The No Observed Effect Concentration (NOEC) was determined by Dunnett's test using the individual replicate values.

Statistical Analysis of Results
The EC50 (0-96 h) with its associated 95% confidence limits of RH-35,201 Crude was calculated using the Probit analysis method (Finney, 1971) and NOEC value through Dunnett's test using computer software.

Result : Test Culture Conditions

The values recorded for pH, temperature and illumination (recorded in lux) were all within the guideline limits.

Test Level mean pH at

	0h	96h
Control	8.5	8.3
Vehicle	8.6	8.3
0.03	8.6	8.5
0.06	8.6	8.6
0.12	8.7	8.6
0.24	8.5	8.4
0.48	8.6	8.4

Observation on day	Temperature (deg C) Minimum	Maximum	Illumination (lux)
1 (0h)	22	23	7400
2 (24h)	22	24	7450
3 (48h)	22	24	7400
4 (72h)	22	24	7450
5 (96h)	22	24	7430

Algal Cell Counts

In the treated and control group, cell growth increased with time and reached a peak by 96 h. Normal cell growth was observed in the control group [72 times in control &71 times in vehicle control] over 96 h.

Test Level	Mean Number Cells/ml at 0h	24h	48h	72h	96h

Control	38333	216667	381250	842917
Vehicle	32083	211667	365833	835417
0.03	30000	203333	349167	801667
0.06	11775	25000	185033	320833	679167
0.12	19167	130833	222500	533333
0.24	12500	500000	115833	207500
0.48	5000	5833	11667	14167

Growth Inhibition, EC50 Values and NOEC

The effect of the RH-35,201 Crude on alga growth was assessed by comparing data obtained from each treatment group with that of the control group. At a concentration of 0.03 mg RH-35,201 Crude/ml least inhibition of growth over vehicle control was observed. At 96 h, concentrations of 0.03, 0.06, 0.12, 0.24 and 0.48 mg RH-35,201 Crude/ml and a vehicle control (without RH-35,201 Crude) recorded 4.46, 15.82, 39.36, 75.57, 101.18 and 0.0 per cent growth (EbC) inhibition and 0.94, 4.83, 10.50, 32.67, 95.74 and 0.0 percent growth rate (ErC) reduction was observed. The EC50 value for RH-35,201 Crude has been determined as 0.131 mg/ml and 0.235 mg/ml for growth (EbC 50) and growth rate (ErC 50) respectively. The 95% confidence limits were 0.07 to 0.243 mg/ml for growth and 0.133 to 0.414 mg/ml for growth rate at 96 h. The no observed effect concentration (NOEC) of RH-35,201 Crude for unicellular alga, Selenastrum capricornutum was found to be 0.03 mg/ml.

Source : The Dow Chemical Company, Midland, MI.

Conclusion : The EC50 value for RH-35,201 Crude has been determined as 0.131 mg/ml and 0.235 mg/ml for growth (EbC 50) and growth rate (ErC 50) respectively. The 95% confidence limits were 0.07 to 0.243 mg/ml for growth and 0.133 to 0.414 mg/ml for growth rate at 96 h.

The no observed effect concentration (NOEC) of RH-35,201 Crude for unicellular alga, Selenastrum capricornutum was found to be 0.03 mg/ml.

Reliability : (1) valid without restriction
01.09.2005

(2)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	> 500 mg/kg bw
Species	:	rat
Strain	:	Crj: CD(SD)
Sex	:	male
Number of animals	:	12
Vehicle	:	other: 0.5% methylcellulose in water
Doses	:	
Method	:	other
Year	:	1978
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Animals fasted for 24 hours were dosed with a 10% (W/V) dispersion of the material in 0.5% methylcellulose in water solution at dose levels of 50 or 500 mg/kg.
Result	:	No animals died prior to study termination. The LD50 was greater than 500 mg/kg. Some diarrhea was observed, but there were no visible lesions at necropsy.
Source	:	The Dow Chemical Company, Midland, MI.
Reliability	:	(2) valid with restrictions Method described is range-finding, but is scientifically valid.

19.05.2003 (2)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC50
Value	:	> 4.13 mg/l
Species	:	rat
Strain	:	Wistar
Sex	:	male/female
Number of animals	:	20
Vehicle	:	other
Doses	:	
Exposure time	:	4 hour(s)
Method	:	OECD Guide-line 403 "Acute Inhalation Toxicity"
Year	:	2001
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Experimental Design

To study the acute inhalation toxicity of RH-35,201 Crude in rats, an experiment was carried out in the inhalation chamber (nose only exposure) supplied by M/S Bio Tox Instrumentations International, New Delhi, India. This dynamic type inhalation exposure equipment consisted of an air compressor, rotameter, jet nebulizer, continuous infusion syringe pump, inhalation chamber, cascade impactor, humitherm, oxygen monitor, rat exposure tubes and flow meter.

The dynamic inhalation chamber has 3 main parts namely inlet, exposure and outlet chambers. Each part is 30 cm in height and 30 cm in internal

diameter. The total capacity of the chamber is 63.5 litres. The inlet unit at top is made up of a glass cylinder with facility for the attachment of a gas inlet tube. The exposure unit (middle) is made up of stainless steel with 20 port-holes to accommodate rat exposure tubes. Rat exposure tubes are made up of a polyacrylic material and provided with orifices to eliminate excreta and urine which are collected in a dropping tray. These exposure tubes are accommodated in the port-holes of the inhalation chamber. The adjustable unit of the exposure tube is set in such a way that animals breathe the test substance aerosol through the window panel of the exposure tube. Observations, during the inhalation experiment were made through transparent exposure tubes. The outlet unit at bottom is made up of stainless steel with an outlet provision connected to a suction pump. The out going air from the chamber passes through the impinger containing 1.0% sodium hydroxide solution.

Procedure

RH-35,201 Crude was loaded into a 60 ml infusion syringe which was positioned on the continuous infusion syringe pump (manufactured by B. Braun Melsungen AG, West Germany). The stock solution concentration was determined to be 1316.55 mg/ml by taking the specific gravity of the test substance.

RH-35,201 Crude was infused into the jet nebulizer where an aerosol was formed and distributed into the inhalation chamber. The calculated nominal concentration of RH-35,201 Crude was 32.914 mg/l air.

The applied air inflow rate was 10 litres per minute while, the outflow was maintained at 11 litres per minute, to ensure slight negative pressure inside the chamber to prevent leakage of the test substance into the surrounding area. The mean chamber temperature and relative humidity during exposures were 21.00C and 41.4 %, respectively. The mean chamber oxygen concentration was 20.0 %.

Each rat was restrained in a single transparent polyacrylic exposure tube with adjustable unit. The exposure tubes were accommodated in the port-holes of the inhalation chamber. The adjustable unit of the exposure tube was set so that the animals breathe aerosols of RH-35,201 Crude released through the window panel of the exposure tube. Observations during the inhalation experiment were made through the transparent exposure tubes.

The control group rats were subjected to a "sham" exposure, to air only. The treated group was exposed for a duration of 4 hours continuously after an equilibration period of 30 minutes.

Gravimetric Concentration Analysis

To assess the breathing zone concentration, a measured volume of air was drawn from the inhalation chamber at the level of breathing zone at every one hour of exposure and determined by the gravimetric method. The open phase sampler with glass microfibre papers was used to assess the breathing zone concentration. At the end of air sampling (5 minutes), the glass microfibre papers with the test substance were weighed to determine the concentration of RH-35,201 Crude aerosols at the breathing zone of the rats.

The particle size was determined by using seven stage cascade impactor.

Description of Seven Stage Cascade Impactor (Model : 02-150)

The unit (manufactured by INTOX PRODUCTS, New Mexico) consists of an inlet cone, seven stages, seven collection plates and a backup filter.

The stages are numbered from 1 to 7 and F. Stage F contains the backup filter. All these impactor stages contain multiple precision drilled orifices. The orifices of the stages are progressively smaller from top to bottom. When air is drawn through the sampler, multiple jets of air in each stage direct any airborne particles towards surface of the collection plate for the respective stage. The base stage has outlet nipple connected to exhaust pump.

Air was led through the cascade impactor (7 stages) with preweighedstainless steel collection plates (coated with 5% silicon grease in hexane). The sampling speed was maintained at 4.12 litres per minute. At the end of air sampling (5 minutes), the collection plates were deassembled and weighed with test substance to determine the concentration of aerosol at the breathing zone of the rats. The increase in the weight of each collection plate was the mass of particles in the size range of that impact stage. The total mass of particles and the per cent mass of particles in each size range were calculated. The mean cumulative per cent particle size was calculated by adding the mean particle size distribution. Mass median aerodynamic diameter was calculated directly from per cent particle size distribution curve.

Toxic Signs

All animals were observed for mortality and any signs of toxicity at hourly intervals during and post 4h exposure period. Subsequently, the animals were observed daily for 14 days, after exposure.

Necropsy

At the end of the study, the surviving animals were sacrificed by cervical dislocation and subjected to gross pathological examination. This consisted of an external examination and the opening of the nasal passage, abdominal and thoracic cavities. The appearance of any macroscopic abnormalities was recorded.

Result : Concentration Details of RH-35,201 Crude in the Inhalation Chamber

The mean concentration of RH-35,201 Crude in the air at the breathing zone of rats was 4.130 mg/l air. The mass median aerodynamic diameter (MMAD) of RH-35,201 Crude was 2.80 5 with geometric standard deviation (GSD) of 2.61 5.

Mortality

No mortality was observed in both the sexes in the control as well as the treated group animals.

Toxic Signs

Animals exposed to RH-35,201 Crude exhibited tremors, abdominal breathing and nasal irritation during the exposure and 4 hour post exposure period.

Abdominal breathing and nasal irritation was consistently observed throughout the exposure period from 2nd to 4th hour. At the end of 4th hour of exposure all animals exhibited tremors.

During the initial post exposure 30 minutes clinical observation period, nasal irritation and tremors were observed in all the animals, except one (14M) which showed symptom of nasal irritation only. Symptom of abdominal breathing was observed in all the female animals during the first 30 minutes of post exposure period. Nasal irritation was observed in two male (11M and 13M) and three female (17F, 18F and 19F) rats at the 1st

hour after exposure. Animals were found to be normal from the 2nd hour of post exposure period.

Body Weight

All the surviving animals showed a gain in body weight over the duration of the experiment.

Necropsy

Macroscopic Findings - External

The external examination of the animals belonging to the control and the treatment groups did not show any significant lesion / abnormality on necropsy.

Internal

On post-mortem examination, majority of animals sacrificed at the termination revealed varying degree of vascular / inflammatory changes with or without emphysema in lungs. Further, lesions were recorded in kidneys (congestion, blotched appearance), Spleen (atrophy, whitish discolouration) and Thymus (haemorrhage) of few animals. The occurrence of these lesions in the treatment group animals was at comparable level, to the control and therefore seemed to be spontaneous / incidental changes, unrelated to the test substance exposure.

Source : The Dow Chemical Company, Midland, MI.
Conclusion : Based on the results of the present study it is concluded that the median lethal concentration (LC50) of RH-35,201 Crude in rats is greater than 4.130 mg/l air at the breathing zone of animals through inhalation route of exposure.
Reliability : (1) valid without restriction
06.05.2003 (2)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Value : > 200 mg/kg bw
Species : rabbit
Strain : New Zealand white
Sex : male
Number of animals : 6
Vehicle : other: material tested undiluted
Doses :
Method : other
Year : 1978
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : The material, as received, was held under an impervious cuff in continuous 24-hour contact with the closely clipped skin.
Result : No animals died prior to study termination. There were no signs of toxicity, although well-defined erythema and slight edema followed by skin desiccation were observed at the application sites. No treatment-related lesions were observed at necropsy.
Source : The Dow Chemical Company, Midland, MI.
Reliability : (2) valid with restrictions
Method described is range-finding, but is scientifically valid.
19.05.2003 (2)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	unspecified
Comment	:	other: Eyes of 3/6 rabbits were washed 20-30 seconds after instillation.
Number of animals	:	6
Vehicle	:	
Result	:	slightly irritating
Classification	:	
Method	:	other
Year	:	1978
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method : 0.1 ml of the material, undiluted, was instilled into the conjunctival sac in the eyes of 6 rabbits. The treated eyes of 3 of the rabbits were washed via irrigation with water ~20-30 seconds after instillation. Eyes were examined for effects on the conjunctiva, cornea, and iris, according to the Draize method, at 24, 48, and 72 hours, and 7 days postdoing.

Result : Results of test:

Time	Structure	Score for Animal 1	2	3	4	5	6	Mean*
24 hrs	Cornea	0	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0
	Conjunctiva	0	4	0	0	2	0	1.3
48 hrs	Cornea	0	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0
	Conjunctiva	0	2	0	0	0	0	0.7
72 hrs	Cornea	0	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0
	Conjunctiva	0	0	0	0	0	0	0
7 days	Cornea	0	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0
	Conjunctiva	0	0	0	0	0	0	0

*The mean value is the average of the scores of animals 1-3 (unwashed eyes).

Source : The Dow Chemical Company, Midland, MI.

Conclusion : Material was mildly irritating to rabbit eyes.

Reliability : (2) valid with restrictions
Method described is range-finding, but is scientifically valid.

22.05.2003 (2)

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	TA98, TA100, TA1535, TA1537
Test concentration	:	0.0001-0.5 microliters/plate for TA1535 and TA1537; 0.0001-7.5 microliters/plate for TA98 and TA100
Cycotoxic concentr.	:	1.0 microliters/plate for TA98 and TA100; 0.5 microliters/plate for TA1535 and TA1537.
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1981
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : RH-35201 Technical (concentrations based on 100% active ingredient) was tested with Salmonella typhimurium strains at the following concentrations: 0.0001-0.5 microliters/plate on TA1535 and TA1537; 0.0001-7.5 microliters/plate on TA98 and TA100. The material was tested using a microsomal enzyme preparation (liver extract from Aroclor 1254 pre-induced rats) as well as a saline buffer control mixture with each strain.

Result : Inhibition of growth was seen in strain TA1535 at a concentration of 0.5 microliters/plate with activation, in strain TA1537 at 0.5 microliters/plate with and without activation, in strain TA98 at 0.5 microliters/plate and above with and without activation, and in strain TA100 at 0.5 microliters/plate and above with and without activation. The material did not demonstrate mutagenic activity when tested following the laboratory's standard procedures.

Strain	TA1535		TA1537		TA98		TA100	
	A	S	A	S	A	S	A	S
Concentration(microliters/plate)								
7.5	-	-	-	-	I	I	I	I
5.0	-	-	-	-	I	I	I	I
2.5	-	-	-	-	I	I	I	I
1.0	-	-	-	-	I	I	I	I
0.1	-	-	-	-	50.0	37.0	93.7	84.0
Diluent Control	-	-	-	-	50.6	38.0	99.4	91.8
S.D.	-	-	-	-	7.2	5.0	9.2	9.6
Anthramine (10 ug/plate)	-	-	-	-	-	-	691.8	105.7
Acetamido-fluorene (50 ug/plate)	-	-	-	-	2188.8	40.5	-	-

Strain	TA1535		TA1537		TA98		TA100	
	A	S	A	S	A	S	A	S
Concentration(microliters/plate)								
0.50	I	28.0	I	I	I	I	I	I
0.10	17.3	24.0	9.3	7.7	50.0	32.7	83.0	91.7
0.01	21.7	31.7	10.7	9.3	41.0	40.7	106.7	112.3
0.001	26.3	32.3	12.3	9.0	47.7	31.3	134.0	117.3
0.0001	27.0	32.3	11.7	11.7	45.0	35.7	102.7	119.0
Diluent Control	24.7	28.2	11.6	10.7	44.9	33.6	107.1	104.2
S.D.	6.4	5.5	3.2	2.3	7.3	5.8	10.4	15.4
Anthramine (10 ug/plate)	295.2	34.0	246.0	12.0	-	-	1078.8	108.0
Acetamido-fluorene								

(50 ug/plate) - - - - 1962.0 32.5 - -
Source : The Dow Chemical Company, Midland, MI.
Conclusion : Results indicate that RH-35201 is not mutagenic in the Microbial Mutagen (Ames) Test.
Reliability : (2) valid with restrictions
The data are judged reliable on the basis of the information provided by the testing facility; full copies of data files were not provided.

19.05.2003 (2)

Type : Ames test
System of testing : TA98, TA100
Test concentration : 3750, 7500 micrograms/plate
Cycotoxic concentr. : 3750 micrograms/plate
Metabolic activation : with and without
Result : ambiguous
Method : other
Year : 1985
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : One lot of RH-35201 was tested as received with Salmonella typhimurium strains TA98 and TA100 at compound concentrations of 3750 and 750 micrograms/plate in the presence and absence of a microsomal activation system (liver extract from Arochlor-induced rats).
Result : Both tested concentrations caused growth inhibition in tester strains TA98 and TA100 both in the presence and absence of a microsomal activation system.
Source : The Dow Chemical Company, Midland, MI.
Conclusion : Since growth inhibition occurred at all tested levels, no conclusion could be drawn.
Reliability : (3) invalid
Since all tested concentrations were inhibitory to growth, no reliable conclusions can be drawn.

19.05.2003 (2)

Type : Ames test
System of testing : TA98, TA100, TA1535, TA1537, TA1538
Test concentration : 0.001 - 5.0 microliters/plate
Cycotoxic concentr. : None
Metabolic activation : with and without
Result : negative
Method : other
Year : 1977
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : MATERIALS

Indicator Microorganisms:
Salmonella typhimurium, strains: TA-1535, TA-98, TA-1537, TA-100, TA-1538
Saccharomyces cerevisiae, strain: D4

Activation System (Ames et al., Mutation Research 31:347, 1975)
1.
Reaction Mixture
Component Final Concentration/ml TPN
4 pmoles
Glucose-6-phosphate 5 pmoles Sodium phosphate
(dibasic) 100 pmoles
MgCl2 8 pmoles
KC1 33 pmoles Homogenate fraction equivalent

0.1-0.15 ml 9,000 x
to 25 mg of wet tissue supernatant of rat liver

S-9 Homogenate
A 9,000 x g supernatant was p@epared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

Positive Control Chemicals
Table I below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1
PROBABLE MUTAGENIC

ASSAY	CHEMICAL a	SOLVENT	SPECIFICITY
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS b
	2-Nitrofluorene (NF)	Dimethyl-sulfoxide c	FS b
	Quinacrine mustard (QM)	Water or saline	FS b
Activation	2-Anthramine (ANTH)	Dimethyl-sulfoxide c	BPS b
	2-Acetylaminofluorene (AAF)	Dimethyl-sulfoxide c	FS b
	8-Aminoquinoline (AMQ)	Dimethyl-sulfoxide c	FS b

a Concentrations given in Results Section
b BPS = Base-pair substitution
FS = Frameshift
c Previously shown to be nonmutagenic

Solvent
Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed and its concentration are recorded in the Results Section.

EXPERIMENTAL DESIGN
A. Plate Test (Overlay Method*)
Approximately 10E8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, at least four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, a minimum of four different concentrations of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and
the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48
hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

*Certain classes of chemicals known to be mutagens and carcinogens do not produce detectable responses using the standard Ames overlay method. Some dialkyl nitrosamines and certain substituted hydrazines are

mutagenic in suspension assays, but not in the platd assay. Chemicals of these classes should be screened in a suspension assay.

Result : See attached document.
Source : The Dow Chemical Company, Midland, MI.
Attached document : ames4.bmp
Conclusion : The test compound, RH-35201, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.
Reliability : (2) valid with restrictions
This study was conducted before the advent of Good Laboratory Practice guidelines, but is scientifically sound.

19.05.2003 (2)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Micronucleus assay
Species : mouse
Sex : male/female
Strain : Swiss
Route of admin. : gavage
Exposure period : 2 consecutive days
Doses : 187.5, 375.0, and 750.0 mg/kg body weight/day
Result : negative
Method : OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year : 2002
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : Test Animals

Fifty (twenty five males and twenty five females) Swiss albino mice of seven weeks age were received from breeding facility, Jai Research Foundation. The mice weighed between 23 and 33 g at the start of the experiment.

Acclimatisation

The animals were randomly divided into five groups each comprising five males and five females and were allowed to acclimatise for a period of 5 days in the experimental room.

Dose Levels

A range finding study for RH-35,201 Crude was performed using four (2 M and 2 F) animals at the dose levels of 500, 1000, 1500 and 2000 mg/kg body weight. Mortality observed were 0%, 0%, 50% and 100%, respectively at the above mentioned dose levels.

The doses of RH-35,201 Crude were selected based on the result of range finding study. The low, mid and high doses employed were 187.5, 375 and 750 mg/kg body weight, respectively.

Treatment

RH-35,201 Crude was suspended in peanut oil at the required concentrations. The test suspensions were administered orally using a metal cannula (size 20 G x 2.75 cm, manufactured by IMS, Dane Mill,Broadhurst Lane, Congleton, Cheshire CW12 1LA, England) attached to a 1 ml Top hypodermic boro-silicate hard glass syringe, to three groups (II, III and IV) of mice for two consecutive days. The test doses employed

were 187.5, 375.0 and 750.0 mg RH-35,201 Crude/kg body weight, respectively, to group II, III and IV. Mice from the control group (I) received only vehicle orally, whereas mice from the positive control group (V) received Mitomycin-C (dissolved in distilled water) intraperitoneally at the dose level of 4 mg/kg body weight, on a single occasion. For all the groups the dose volume was 10 ml/kg body weight.

Body weight was recorded before dosing on each day and prior to sacrifice. The clinical signs of toxicity were recorded after dosing and before sacrifice.

Slide Preparation

The day following the last treatment, mice from all the groups were sacrificed by cervical dislocation. Femora from the sacrificed animals were excised and the epicondyle tips removed. The bone marrow content was expelled by flushing and aspiration using a 1 ml syringe and 24 gauge needle into centrifuge tubes along with 3 ml of fetal calf serum. The aspirated bone marrow content was mixed using the syringe to dissociate the cells and also to avoid cell clump formation. The tubes were centrifuged at 2000 rpm for 10 minutes and supernatant was discarded leaving about 0.5 ml of the medium with cell pellet. The cell pellet was dissociated thoroughly using pasteur pipette and a drop of the serum with suspended cells was placed on a clean slide marked with study number, animal number and slide number. A smear was prepared and allowed to air dry. Two slides were prepared per animal and the cells were fixed with absolute methanol and allowed to air dry. Slides were stained using 5% Giemsa in phosphate buffer (pH 6.8) for 10 minutes. Subsequently the slides were rinsed in distilled water and air dried. In order to prevent bias in the scoring procedure, the slide numbers were masked with code numbers provided by Department of Bio-statistics and Systems Information, JRF.

Scoring Bone Marrow Micronucleus

The coded slides were examined for the presence of micronuclei in polychromatic and normochromatic erythrocytes under a microscope (Nikon Optiphot-2). A minimum of 2000 polychromatic erythrocytes were screened per animal and the incidence of micronuclei was recorded, the corresponding number of normochromatic erythrocytes with and without micronuclei were also recorded. Out of the two slides prepared per animal, one slide was used for screening micronucleated erythrocytes whereas the other slide was kept in reserve. The masked labels were removed after scoring all the slides. The per cent micronucleated polychromatic erythrocytes and the ratio between total polychromatic erythrocytes (PCE) and total erythrocytes was calculated.

Statistical Evaluation of Results

The data of body weight, per cent micronucleated polychromatic erythrocytes and P/E ratio were statistically analysed by using the Student's "t" test (Gad and Weil, 1994).

Result : Body Weights

Following two days oral dosing of RH-35,201 Crude, no significant change in body weight of male and female mice were observed up to a dose level of 750.0 mg RH-35,201 Crude/kg body weight.

Clinical Observations

Day 1
Three animals (11M, 16F and 17F) of group II and one animal (23M) of group III exhibited tremor. One animal (37F) of group IV exhibited lethargy.

One animal (12M) of group II exhibited both lethargy and piloerection whereas one animal (31 M) of group IV exhibited tremor and piloerection. Two animals of (14M and 20F) of group II, three animals (21M, 26F and 29F) of group III and three animals (35M, 38F and 39F) of group IV exhibited piloerection.

Day 2
Tremor was observed in three animals (11M, 15M and 18F) of group II, one animal (29F) of group III and three animals (32M, 38F and 39F) of group IV. Piloerection was observed in two animals (12M and 17F) of group II, three animals (23M, 24M and 26F) of group III and two animals (33M and 36F) of group IV. One animal (22M) of group III and four animals (31M, 34M, 37F and 40F) of group IV exhibited both tremor and piloerection.

All the other animals were found to be normal throughout the study period and prior to sacrifice except animal number 31M of high dose (group IV), which was found dead on the day of sacrifice.

Micronucleated Polychromatic Erythrocytes

The per cent micronucleated polychromatic erythrocytes in bone marrow cells of male and female mice treated with RH-35,201 Crude up to a dose level of 750.0 mg/kg body weight did not show any significant change from that of the control. The P/E ratio also did not significantly vary from that of the control in both the sexes treated up to 750.0 mg RH-35,201 Crude/kg body weight. The bone marrow smears of mice treated with RH-35,201 Crude did not have any abnormal figures such as lagging chromosome and large sized micronuclei. The results of positive control (4 mg Mitomycin-C/kg body weight) proved the sensitivity of micronucleus test in mice.

Interpretation of Results

Based on the results obtained from the present investigation under these test conditions, RH-35,201 Crude does not have micronucleus induction potentiality in the bone marrow cells of male and female mice up to the dose level of 750 mg/kg body weight. No effect level observed was 750.0 mg RH-35,201 Crude/kg body weight.

Source : The Dow Chemical Company, Midland, MI.
Conclusion : From the above study, it is concluded that RH-35,201 Crude does not have micronucleus induction potential in male and female mice following two days oral dosing up to the dose level of 750.0 mg/kg body weight. No effect level observed was 750.0 mg RH-35,201 Crude/kg body weight.
Reliability : (1) valid without restriction
06.05.2003 (2)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type : other
Species : rat
Sex : male/female
Strain : Wistar
Route of admin. : oral feed
Exposure period : From 14 days prior to the mating, during the mating period, during the resultant pregnancies and through the lactation day 4. Treatment continued throughout the study till one day prior to sacrifice.
F015 Continious through diet

Frequency of treatm.	:	Continuous in diet
Premating exposure period		
Male	:	14 days
Female	:	14 days
Duration of test	:	~7 weeks
No. of generation studies	:	
Doses	:	0, 500, 1500, 4500 ppm
Control group	:	yes, concurrent vehicle
NOAEL parental	:	= 500 ppm
NOAEL F1 offspring	:	= 4500 ppm
Method	:	OECD combined repeated dose and reproductive/developmental toxicity screening test
Year	:	2002
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Experimental Design

The study consisted of three treatment groups (500, 1500 and 4500 ppm) and a concurrent control group. Each group consisted of 12 males and 12 females which were housed separately by sex (three per cage) and were given the respective diets for 2-weeks. After two weeks of pre-mating treatment, one male and one female of the respective treatment group were cohabited for a period of subsequent 14 days or till the females were sperm positive, whichever happens first. Each morning during the co-habitation period, the females were examined for the presence of sperm in their vaginal smear. Females showing evidence of copulation (presence of sperm in their vaginal smear) were assigned as day "0" of pregnancy (gestation day "0") and these females were separated from their respective male and observed till the resultant lactation. Females showing no evidence of copulation and those presumed pregnant but did not deliver were euthanised by CO2, 26 days after the last day of the mating period. All pups were observed daily. All pups were sent for necropsy on day 4 of lactation. Subsequent to the necropsy of the pups, adult males and females (5 per group) were subjected to sensory reactivity to stimuli, motor activity, haematology, clinical chemistry and urinalysis. Detailed clinical observation was conducted weekly on all male and female animals till they were sacrificed.

Route of Administration

The route of administration was oral, through diet. The test substance was administered at a constant concentration (ppm) in animal's diet.

Frequency of Diet Preparation

Based on the stability of RH-35,201 Crude in diet, the experimental diet was prepared once in three or four days throughout the experimental period.

Experimental Diet Preparation

Experimental diets for each group of animals were prepared in quantities of 4 kg, per group per mixing. The requisite quantity of RH-35,201 Crude was obtained from the JRF, Test Substance Control Office (TSCO) and was then added to the powder diet (in case of control group, only feed was used) to produce the requisite test diet. Mixing was performed with a `Y'-shaped blender (manufactured by M/s. Sagar Pharma Industries, Mumbai, India, maximum capacity of 10 Kgs.) for 20 minutes and the resulting test diet duly transferred to polythene bags within labelled stainless steel containers and stored in the experimental room. The experimental diets

were tested at regular intervals for homogeneity of mixing.

Frequency of Administration

The diet mixed with various concentration of test compound RH-35,201 Crude was made available to the rats ad libitum for seven days a week basis.

Observations

Clinical Signs

Animals were observed for signs of toxicity such as skin and fur changes, eye and mucous membrane changes, respiratory, circulatory, autonomic and central nervous system, somatomotor activity, behaviour pattern and general changes once a day. Physical examination was carried out on all animals prior to the initiation of exposure and at weekly intervals thereafter.

Detailed Clinical Observations

The detailed clinical observation of each adult animal for the following parameters were evaluated and recorded prior to initiation of exposure and at weekly intervals thereafter. These observations using scoring system was made outside the home cage in a standard arena and around the same time of the day, each week.

Open Field Measurements

The test animals were placed in an open field arena (outer dimensions 50.7 x 50.7 x 20.3 cm) and the following parameters evaluated.

Handling Reactivity

While handling the animals the following observations were recorded:

Classification	Description
Very easy	Rat does not show any sign of discomfort
Easy	Alert, limbs put against the body
Moderately easy	Vocalization without resistance
Freezes	Rigid in hand
Difficult	Squires, twists, attempts to bite

Palpebral Closure

The degree of closure of the eyelids during the time when the animal was held by the observer, was recorded as wide open, slightly closed or completely closed.

The degree of closure of eyelids was recorded as :
Eyelids wide open
Eyelids slightly closed
Ptosis (drooping of eyelids)
Eyelids completely closed

Lacrimation

The degree and nature of lacrimation was recorded as present or absent.

Classification	Description
None	No external lacrimation

Slight	Wetness of lower eyelids
Severe	Dropping of tears

Eye Examination

The eyes were observed for normal or presence of microphthalmia, exophthalmia, cataract, opacity, chemosis, conjunctivitis, discharge etc.

Piloerection

The presence or absence of piloerection was recorded, conventionally. Piloerection was differentiated from a scruffy or ungroomed coat by patting the back of the animal in a rostral to caudal direction. Piloerection was considered present if the animal's hair remained erect after patting.

Skin Examination

The changes in skin such as rough coat, alopecia and dermatitis were recorded (if any).

Salivation

The degree of salivation was recorded as none, slight or severe.

Classification	Description
None	No external salivation observed
Slight	Wetness of lower mandibular region
Severe	Dropping of saliva

Gait

The manner of walking of the animal was evaluated by observing ambulatory movements in the open-field box. Any abnormalities observed was ranked in severity as normal, slightly abnormal, or severely abnormal.

Mobility

The animal was evaluated for movement such as normal, slightly impaired or totally impaired.

Arousal

A measure of the ability of the animal to locomote despite gait abnormalities was recorded. The ranking of the degree of arousal was recorded as very low, low, high or very high. This value was usually equal to the gait score.

Vocalization

The occurrence of spontaneous or unprovoked vocalizations audible to human ear was recorded. Spontaneous vocalizations were considered those, which were not elicited by handling or other stimulation.

Rearing

The number of times the animal raises its front feet off the floor was considered rearing. The number of these actions was counted for the observation period and the total number of rearings were recorded.

Respiration

The presence or absence of apparent alteration in rate and/or ease of respiration such as abdominal breathing, gasping, snuffles was recorded by observation.

Clonic / Tonic Movements

Clonic movements were those marked by alternate contraction and relaxation of muscles. Tonic movements are defined as a state of continuous muscular contraction. Although clonic and tonic movement may occur together, the presence of each was recorded independently. The following observations were made for clonic/tonic movements :

Clonic Movements
Chewing, clonus of the jaw
Mild clonic tremors of limbs
Repetitive clonic tremors

Tonic Movements
Tonic contraction of hind limb
Ophisthotonus - backward
Emprosthotonos - forward

Urination and Defecation

The number of urine pools and /or fecal boluses on the paper at the end of the observation period was recorded. Polyurea and/or diarrhea were recorded, if present.

Stereotypy and Bizarre Behaviour

Stereotypy was defined as the pronounced repetition of specific gestures or movements, i.e., the presence of excessive or repetitive behaviour that appear purposeless to the observer. Bizarre behaviour includes any unusual behaviour that were observed in the test species.

The presence or absence of stereotypy or bizarre behaviour [such as retropulsion, biting, biting of cage, self destructive biting (bite marks on the tail, paw etc.) or self mutilation etc.] if any were recorded.

Once during the study, usually the week before terminal sacrifice, the following parameters were studied.

Motor Activity

Total and ambulatory activity for five randomly selected animals per sex per dose group was evaluated in an animal activity measuring system (Columbus Instruments, Ohio) equipped with a computer analyser. Animals were monitored for 3 consecutive ten minute intervals allowing for examination of both exploratory and acclimation activity levels. Stereotypic activity was calculated by subtracting ambulatory activity from total activity.

Sensory Reactivity of Stimuli

Once during the study, sensory reactivity to stimuli of different modalities, were carried out in five male and five female experimental animals randomly selected from each dose group.

In males these functional observations were made after mating and towards the end of the treatment period (28 day), shortly before the scheduled sacrifice, but before the blood sampling for haematology and clinical chemistry. The female experimental animals were evaluated after the pups were euthanised.

The following observations were made :

Click Response

A clicker (eg. a child's clicker) was positioned approximately 5 cm above the back of the animal with care taken not to have the clicker in the animal's field of vision. The clicker was held in the palm of the hand to ensure consistency of sound from test to test. The degree of the elicited response was recorded as absent or present.

Touch Response

Approaching the animal from the side, the rump of the animal was gently touched with a blunt object (eg. the blunt end of a pen/pencil). The contact was brief (approximately 2 to 4 seconds) and deliberate but not forceful. The degree of the elicited response was recorded as absent or present.

Tail-pinch Response

The tail was squeezed approximately 2 to 3 cm from the tip using forceps, without bruising (always applying approximately the same amount of force with each animal). The degree of the elicited response was recorded as flinch, slight reaction or no reaction.

Approach Response

The animal was approached at nose level with the end of a blunt object (eg. the blunt end of a pen/pencil), which was held approximately 3 cm from the face of the animal for approximately 4 seconds to allow time for the animal to respond. Care was taken so as to not touch the vibrissae. The degree of the elicited response was recorded as absent, slow, or fast response.

Pupil Response

The beam of a pocket-sized flashlight was brought from a lateral position medially towards the centre of the face of the animal. Constriction of the pupil was observed as a positive response. The degree of elicited response was recorded as normal, slight or absent.

Air Righting Reflex

The animal was held in the supine position, with the hands of the observer under the back and shoulders of the animal for support. The animal was dropped from a height of approximately 30 cm. The landing was recorded as normal, uncoordinated or lands on back.

Hind Foot Splay

The fourth digit pad of both hindlimb of each rat was inked with a non-permanent, non toxic ink (Indian ink) immediately prior to testing. The animal was suspended in a prone position approximately 30 cm above the testing sheet. The animal was released, and after landing, the distance between the ink print of the toes of the hindlimb was measured on the testing sheet. Three trials were conducted, and the average of the 3 measurements was recorded.

Grip Strength

Grip strength of both forelimb and hindlimb was measured with a grip strength meter (Columbus Instruments, Ohio) to determine the ability of the

animal to grasp and hold on the mesh platform. The grip strength of each animal was measured for 3 consecutive times, and the mean value was calculated separately for the forelimbs and hind limbs.

Body Weights

All the experimental rats were weighed on the first day of treatment, weekly thereafter and at termination. Pregnant animals were weighed on days 0, 7, 15, and 20 of the gestation period, while the lactating female animals were weighed on days 1 and 4 of the post-partum period. Parturition day 1 (P1) was defined as the day on which the female experimental animals litter (weight was recorded within 24 hours of parturition).

Feed Consumption

The cage-wise feed consumption was recorded weekly throughout the experimental period [exclusive of the mating (cohabitation) period] and the weekly feed consumption per cage was calculated. During gestation period, feed consumption was measured on the same day as weighing of the pregnant animals. After parturition and during lactation, feed consumption measurements were made on the same day as weighing of the litters. Feed spillage/waste was not estimated during the course of the study and evidence of excessive spillage was documented in the raw data.

Pup Observations

Litter-wise pup observations were made along with individual animal observations. Each litter was examined as soon as possible after delivery to establish the number and sex of pups, runts, stillbirths, live births and the presence of gross anomalies.

Pup Body Weights

Body weights of pups/litter was recorded on days 1 and 4 after birth.

Clinical Pathology Observations

Clinical pathology tests were conducted on blood samples collected from randomly selected 5 males and 5 females per group animals at the end of the test period. Animals were fasted overnight and blood samples were collected by puncturing the orbital sinus with the help of a fine capillary tube under ether (anaesthetic) anaesthesia. For determination of clotting time, blood was allowed to flow into a 7.5 cm capillary tube and the time required for clotting was recorded manually. Around 0.5 ml of blood was collected in vials containing EDTA for haematology analysis. One drop of blood was taken on clean glass slide, spread and stained with Leishman's stain for differential leucocyte count.

Two to three ml of blood was collected from each animal in clean centrifuge tubes for serum separation. The blood was allowed to clot at room temperature and the serum was separated by centrifugation at low speed. Serum was stored under frozen condition while not in use.

The following haematological and clinical chemistry parameters were studied at the end of treatment period on blood samples collected by orbital sinus puncture from five animals per sex of all the dose groups.

Parameter	Sample Type	Instrument used
Haematology		
Leucocyte count (WBC)	WB	Sysmex K1000

Erythrocyte count (RBC)	WB	Sysmex K1000
Haemoglobin (HGB)	WB	Sysmex K1000
Haematocrit (HCT)	WB	Sysmex K1000
Mean corpuscular volume (MCV)	WB	Sysmex K1000
Mean corpuscular haemoglobin (MCH)	WB	Sysmex K1000
Mean corpuscular haemoglobin concentration (MCHC)	WB	Sysmex K1000
Platelet count (PLT)	WB	Sysmex K1000
Clotting time	WB	Manual
Reticulocyte count	WB	Manual
Prothrombin time	Plasma	Manual
Differential leucocyte count	WB	Manual

Clinical chemistry

Calcium	Serum	ERBACHEM-5PLUS
Phosphorus	Serum	ERBACHEM-5PLUS
Chloride	Serum	ERBACHEM-5PLUS
Sodium	Serum	Flame photometer
Potassium	Serum	Flame photometer
Glucose	Serum	ERBACHEM-5PLUS
Cholesterol	Serum	ERBACHEM-5PLUS
Aspartate aminotransferase (SGOT)	Serum	ERBACHEM-5PLUS
Alanine aminotransferase (SGPT)	Serum	ERBACHEM-5PLUS
Gamma glutamyltranspeptidase (GGT)	Serum	ERBACHEM-5PLUS
Urea and Blood urea nitrogen (BUN)	Serum	ERBACHEM-5PLUS
Albumin	Serum	ERBACHEM-5PLUS
Creatinine	Serum	ERBACHEM-5PLUS
Total bilirubin	Serum	ERBACHEM-5PLUS
Total proteins	Serum	ERBACHEM-5PLUS
Alkaline phosphatase	Serum	ERBACHEM-5PLUS

Key: WB = whole blood

Urine Analysis

Urine analysis were performed for 5 male and 5 female rats from each dose group before terminal sacrifice. The parameters viz., appearance, volume, specific gravity, pH, protein, glucose and blood/blood cells were analysed semi-quantitatively.

Terminal Observations

Gross pathological observations were made for all the experimental animals. Animals were subjected to a complete gross necropsy including examination of external surfaces, orifices, cranial, thoracic and abdominal cavities and their contents. The necropsy was performed by or under the supervision of a veterinary pathologist.

The testes and epididymides of all adult male rats were weighed and then fixed in Bouin's fixative for histopathology evaluation.

The female rats have the uteri examined at necropsy for the presence of implantation sites and resorptions and the corpora lutea counted. Also, the uteri of apparently non-pregnant female experimental animals were examined grossly.

The preserved organs from control and high dose animals were processed

following suitable technique of washing by tap water, dehydration in ascending grades of isopropyl alcohol, clearing in xylene, embedding and blocking in paraffin wax. Paraffin sections (4 to 5 5m) were cut, processed and mounted on slides, strained by haemoglobin and eosin and examined microscopically.

Full histopathological examination of the above listed organs were performed for all the control and high-dose group animals, with special emphasis on stages of spermatogenesis in the male gonads and histopathology of interstitial testicular cell structure. In case, no treatment-related effect/s were observed in the high dose animal's, subsequent lower dose groups were not examined.

The weights of adrenal, brain, thymus, testes/ovaries, epididymis/uterus, heart, kidneys, liver and spleen were recorded for all the experimental animals and relative organ weight was calculated.

Dead pups and pups killed on day 4 post partum were carefully examined externally for gross abnormalities.

Evaluation of Data

The data on the number of sperm positive (pregnant) animals in each group and number of animals kept for mating were compiled and the pregnancy rate was expressed in terms of percentage. The mean of litter size and weights, was calculated for the control and treated groups.

Statistical Analysis

Raw data were processed by the Department of Biostatistics and Systems Information, Jai Research Foundation to give group means and standard deviations with significance between the control and treated groups, using in-house developed statistical software. All the parameters characterised by continuous data such as hindlimb foot splay, grip strength, motor activities, rearing count, urination count, defecation, body weight, feed consumption, organ weight, relative organ weight, haematological and clinical chemistry data were subjected to Bartlett's test to meet the homogeneity of variance before conducting Analysis of Variance (ANOVA) and Dunnett's t-test. Where the data did not meet the homogeneity of variance, Student's t-test was performed to calculate significance.

Remark : Type of test was the combined repeated dose toxicity with reproduction/developmental toxicity screening.

Result : No treatment related effects in mortality, clinical symptoms or detailed clinical observations were recorded in any of the treatment groups. No effects on body weight were observed at the low and mid dose levels of either sex. However, treatment related decrease in body weight was observed at the high dose (4500 ppm) in both males and females (during all phases of study).
Fertility, reproductive and lactation indices were comparable across all treated groups. Pup body weights were comparable at the low and mid dose with the control. However, pup body weights were significantly lower during lactation at the high dose (4500 ppm).
A dose related increase in liver weight was apparent in all treated groups. This was considered treatment related. Although, no concomitant histopathological treatment related effects were present in the livers of the treated animals. Gross and histopathological examination did not reveal any treatment related lesions at any of the treatment levels.

Based on the above findings, the No Observed Effect Level (NOEL) for reproductive effects is concluded to be 4500 ppm (the highest dose employed). However, the No Observed Adverse Effect Level (NOAEL) for the systemic toxicity (repeated dose) for both the adult and pups, males

and females was considered to be 500 ppm.

Mortalities

No mortalities were observed in adult animals of either sex from the control and RH-35,201 Crude at the dose level of 500, 1500 and 4500 ppm during the course of the study.

Clinical Observations

No treatment related visible signs of toxicity were observed in adult animals of either sex in the control and treated with RH-35,201 Crude at 500, 1500 and 4500 ppm.

Detailed Clinical Observations

Male

The detailed clinical observations were recorded for all animals prior to treatment and at weekly intervals, thereafter.

Prior to exposure and during weekly intervals thereafter, majority of the animals scored "easy" and few rats in 1500 and 4500 ppm dose group scored "very easy" and "moderately easy" for handling reactivity.

The palpebral closure was recorded as "wide open" for all the rats of control and treatment groups throughout the study period.

All the rats from all the groups scored "none" for lacrimation, "normal" for eye examination and skin examination; "none" for salivation and, "absent" for piloerection till the end of experimental period.

No lacrimation, salivation or piloerection was recorded during the course of the study in RH-35,201 Crude treated or control animals.

Prior to treatment, all the animals gait and mobility was observed as normal. During the first week of experiment, one animal showed slight abnormality at 500 ppm dose group. During the fourth week of experiment one animal from control and two animals from high dose (4500 ppm) group showed slight abnormality. During the week 2 and 3 all animals showed gait as normal. Since the gait abnormality was observed to be sporadic and transient in nature, it was concluded to be incidental.

Arousal was recorded "high" for majority of the animals and "low" for few animals irrespective of dose group and "very high" for few animals at 1500 ppm treated group during the course of the study. Vocalization was not observed for any animal irrespective of dose group till the end of the study.

Rats from low (500 ppm) dose group showed statistically significant increase in the values of mean rearing counts during 1st week and rats from low and mid dose (500 and 1500 ppm) groups showed reduction in rearing count on week 2 as compared to control group. Further, the rats from high dose (4500 ppm) group showed increased rearing values but mid dose (1500 ppm) group showed decreased value as compared to control group. As no definite pattern was observed these findings were deemed incidental.

All the animals from all the dose groups scored "normal" for respiration and "absent" for bizzare behaviour throughout the study.

Neither clonic nor tonic convulsions were observed in the animals from the control and treated dose groups during the experimental period.

No alterations in the values of mean number of urine pool was observed in 500 and 1500 ppm treated groups as compared to control. At 4500 ppm dose level significant increase in number of urine pool was observed only during the week 1 and 3 of the experimental period.

Statistically significant decrease in the values of mean number of fecal boluses was observed from all treated dose groups on week 4 as compared to the control. The fecal pellet count values for high dose animals were significantly less during the pre exposure period, week 1 and 2 when compared to control group.

Stereotypy was recorded as "Absent" for majority of the animals and excessive grooming for few animals in the treated dose groups from week 2, 3 and 4 during the experimental period.

Based on the above finding it is apparent that no consistent dose or treatment related changes in clinical observations were apparent in the rats from treated groups, when compared with the concurrent control groups.

Female

Prior to the treatment and at all subsequent intervals, majority of the rats showed "easy" for handling reactivity, while few animals showed "moderately easy" during handling.

Irrespective of treatment period, palpebral closure of rats from all the dose groups was recorded as "wide open" during the entire period of the study.

Lacrimation and piloerection were not seen in any rat from any group during the course of the study.

Eye and skin appeared normal for all animals during the entire experimental period.

No salivation was observed in the control, 500 and 1500 ppm treated group animals. At 4500 ppm treated group, two female animals exhibited slight salivation during pre exposure period and throughout the experimental period.

Gait and mobility were recorded as "normal" for all female rats during experimental period.

Majority of the rats from various treatment groups showed "high" arousal throughout the study. Few rats showed "low", "very low" and "very high" (only in low dose group) arousal during few weekly interval of the study.

Vocalization was not observed from any animals during the experimental period.

The mean values for rearing counts were significantly decreased during 2nd week, from the treated dose of 500 and 4500 ppm group animals. However, statistically significant decrease in 1500 ppm group and increase in 4500 ppm dose groups was observed during pre-exposure observation. There is no significant change observed at 1500 ppm treated dose group.

All the animals from control and treated dose groups scored "normal" for respiration, "absent" for clonic movement, tonic movement, bizarre behaviour and stereotypy (except one rat showed excessive grooming on 5th week of experiment at 1500 ppm dose group).

No alteration in the mean value of number of urine pools and fecal boluses

was observed in the treated group when compared to control group, except significant decrease in urine pool was observed at 500 ppm dose group during the experimental week "5".

Based on the above finding it is apparent that no definite dose or treatment related changes in clinical observation / parameters were apparent in any of the treated groups.

Sensory Reactivity to Stimuli

Male

All rats from control and 500 ppm dose groups showed presence of touch response to stimuli. One rat from 1500 and 4500 ppm dose groups showed absence of touch response. Click response was absent in one rat of 500 ppm, two rats of 1500 ppm and one rat of 4500 ppm dose group. Majority of the rats exhibited tail pinch response as flinch, however, one rat each at low and mid dose treated groups showed slight reaction for tail pinch response. Pupil response and air righting reflex of all rats were found to be normal. All rats from control and treated dose group showed "fast" approach response, except one rat showed "absent" at 4500 ppm dose group. These observed changes were not considered to be treatment related.

Female

Touch response to stimuli was recorded as absent in one rat at 500 ppm dose group and other rats were exhibited as present for touch response. Click response was absent in one rat each from control and 1500 ppm dose group, other rats showed presence of click response in control and treated groups. All the rats recorded tail pinch response as flinch, during the experimental period. Pupil response and air righting reflex of all the rats were found to be normal during the observation. Majority of the rats from treatment and control groups revealed fast approach response during the experimental period. The observed changes were not considered related to test substance.

Motor Activity

Male

There were no treatment related alterations in the motor activity between RH-35,201 Crude treated groups and control group during the experiment. Total activity counts of animals from high dose 4500 ppm, were significantly increased during the second ten minutes interval (i.e. 11 - 20 minutes) of observation and stereotypic activity count during 21 - 30 minutes at 1500 ppm dose group. Such changes were not observed at other time intervals for the 1500 and 4500 ppm males or in the animals of the other dose groups, these changes were deemed incidental.

Female

Treatment of RH-35,201 Crude did not cause significant alterations in motor activities as compared to control. However, significant increase in stereotypic activity during 11 - 20 minutes was observed in animals from mid dose group (1500 ppm) as compared to control. Such changes were considered incidental because they were not observed at other time intervals for the 1500 ppm females or in any other treatment groups.

Grip Strength

Male

No significant alteration were observed in forelimb and hindlimb grip strength of RH-35,201 Crude treated groups as compared to the control group.

Female

Administration of RH-35,201 Crude did not exhibit any significant change in forelimb and hindlimb grip strength as compared to the control.

Foot Splay

Male

Administration of RH-35,201 Crude did not exhibit any significant effect on hindlimb foot splay.

Female

No significant alteration in hindlimb foot splay of female rats administered with RH-35,201 Crude was observed.

Body Weight

Male

No significant effect on body weight was observed at 500 and 1500 ppm dose during the treatment period. However, at 4500 ppm a significant decrease was observed in the weekly body weight during the second week (cohabitation period) of the experiment. In subsequent weeks also, the body weight of 4500 ppm dose group males remain depressed, although not statistically significant. In the absence of any significant decrease in feed consumption, the decrease in the body weight in the 4500 ppm dose group male was considered treatment related.

Female (Premating, Gestation and Lactation)

The body weight at 500 and 1500 ppm dose level remained comparable with the control group throughout the study period. However, at 4500 ppm a consistent pattern of lower body weight was observed at all time points, although it was statistically significant only during day 15 and 20 of gestation and day 1 of lactation. In light of the increased food consumption in the high dose group (4500 ppm) females, a significant decrease in body weight was considered treatment related.

Feed Consumption

Male

No significant alterations in feed consumption was recorded in male rats from any of the treated groups compared to control, except significant decrease at 1500 ppm dose group on experimental week one only. In the absence of any pattern, this effect was considered incidental and not treatment related.

Female (Premating, Gestation and Lactation)

No significant effect on feed consumption was observed at 500 and 1500 ppm dose at any time during the treatment period. However, at 4500 ppm a consistent pattern of increased feed consumption was observed throughout the treatment period. This increase was considered treatment related, particularly in the presence of a decrease in the body weight of these

females.

Average Test Substance Intake

Test substance intake was calculated from feed consumption, dose and body weight values. The group mean test substance consumption for males was (low dose) 43; (mid dose) 121; and (high dose) 429 mg/kg b. wt./day and for females was (low dose) 55; (mid dose) 147; and (high dose) 668 mg/kg b.wt./day during premating period.

The group mean test compound consumption for gestation females was (low dose) 54; (mid dose) 149; and (high dose) 1177 mg/kg b.wt./day and for lactating females was (low dose) 32; (mid dose) 84; and (high dose) 486 mg/kg b.wt./day.

Fertility Data

Fertility index (male and female) and gestation indices were comparable between the control and all treated groups. Pregnancy rate and parturition indices were not affected by the treatment. The duration of the gestation period was comparable among all the groups. Number of corpora lutea, pre-implantation loss and post-implantation losses were comparable among all groups.

The live birth index, survival index and lactation index were comparable among all treated groups.

Significant decrease in mean number of implantations was observed only at 1500 ppm dose level when compared to the control group. This change was not observed in other treatment groups.

Litter Observation

Litter Size and Litter Weight

Litter size and litter weight were recorded on post partum days 1 and 4. Male and female pup number were comparable in all treated dose groups. Male and female pup weights were comparable at 500 and 1500 ppm dose groups as compared to the control. However, a significant reduction in the pup weight was observed in both sexes at days 1 and 4 in 4500 ppm dose group. This was also reflected in decreased maternal body weights recorded during gestation in 4500 ppm dose group.

Litter Mortality

No significant increase in number and per cent mortality observed in the treated dose groups between lactation days 1 to 4. One pup mortality was observed in control, two pups mortality in 500 ppm group and 14 pups mortality at 4500 ppm dose during the lactation days 1 to 4. In high dose group all pups were cannibalized / found dead on lactation day 3 only in one litter (dam N0 87). Sporadic incidences of cannibalism has been noted historically for Wistar strain of rats bred at JRF.

Haematology

Haematological parameters of all the RH-35,201 Crude treated groups were found to be comparable with the control. However, a statistically significant decrease in MCV was observed in mid dose group and HCT of high dose group males when compared to control. The MCH values were significantly increased in low dose group and decreased in high dose group female as compared to control. Significant increase in lymphocyte value of female was observed at 1500 ppm dose group only. These

observed changes were not considered as treatment related, since they did not follow any particular pattern.

Clinical Chemistry

Most of the clinical chemistry parameters evaluated did not show any significant effect when compared to control. Inconsistent, statistically significant variations were observed in BUN and urea (an increase at the low dose females only), cholesterol (a decrease at the low and mid dose females only) and phosphorus levels (a decrease in mid dose males only). A significant increase in serum sodium level was observed in all treated males but not in females. However, no concomitant changes were observed in serum potassium levels either in males or females from any treatment groups. The statistically significant increase in serum sodium level without a concomitant alteration in potassium level is inconsistent with the physiology, hence it was considered not treatment related.

Urine Analysis

No treatment related effects were observed in any of the parameters studied, with the exception of the pH. The urinary pH was decreased in all treated group, males only. Absence of similar decrease in urinary pH in females which consumed more amount of the test compound and lack of any histopathological effect in kidneys of treated animals at any level suggests that the decrease in urinary pH in males may be incidental.

Terminal Studies

Organ Weight

With the exception of the liver and heart weights, all other organ weights were comparable between the control and various treated groups. The absolute weight of heart in all treated males and only in high dose females were decreased, but the relative heart weights were comparable to control group. Hence, the lower absolute heart weight observed is not considered treatment related. A significant increase in liver (both absolute and relative) weight was observed in most treatment groups. The effect was more pronounced in males than in females, even though females consumed more test compound in each of the group per unit weight of the animal. In spite of a significant increase in liver weight in most treated groups, there were no histopathological changes apparent in the treated rats.

Pathological Findings

Macroscopic Findings

Males

On post-mortem examination, carcasses from different groups revealed varying degree of lesions in lungs (congestion, haemorrhage, pneumonic changes, adhesion with thoracic wall); kidneys (congestion); liver (abscess); spleen (whitish discolouration) and intestine (mucus exudation). No treatment related dose dependent changes were observed.

Females

The changes observed were similar to those observed in males. Additional changes were congestion in liver and emphysema in lungs. No treatment related, dose dependent changes were observed.

Microscopic Findings

Males

On microscopic examination of various organs, varying degree of histopathological changes were seen in animals belonging to control and treatment groups : lungs (congestion, haemorrhage, mycoplasmosis, Clara cell hyperplasia, alveolar histiocytosis, bronchiectasis, medial hypertrophy of muscular arteries, hyalinization); trachea (cystic dilation of submucosal glands, mononuclear cell infiltration); liver (degenerative changes); heart (chondroid metaplasia, pericarditis, myocarditis, perivascular lymphocytic infiltration); kidneys (congestion, cyst, mononuclear cell infiltration, tubular cast, hyperplasia of transitional cell of renal pelvis); urinary bladder (seminal coagulum, mononuclear cell infiltration, cut section of parasite); spleen (congestion, lymphoid hyperplasia, pigmentation, extramedullary haematopoiesis); brain (Buscaino bodies, gliosis, ependymal cell hyperplasia); pituitary (haemorrhage, cyst); adrenals (congestion, accessory cortical nodule, cyst); lymph node (lymphoid hyperplasia); thymus (haemorrhage, epithelial hyperplasia); gastro-intestinal tract (parakeratosis, lymphoid hyperplasia /aggregates, goblet cell hyperplasia, cut section of parasite); testes (atrophic changes, exfoliation / degeneration of germ cell, cellular debris); epididymis (cellular debris, oligospermia, vacuolation / clear cell change); seminal vesicle / coagulating gland (hyperplasia of lining epithelium) and bone-marrow (femur - myeloid cell hyperplasia).

Females

The histopathological changes observed in different viscera were similar to the lesions recorded in male counterparts. Additional changes were recorded in trachea (hyperactive submucosal glands); kidneys (degenerative changes); brain (congestion, ventricular dilatation); pituitary (Rathke's cleft with secretion, hyperplasia of pars intermedia); lymph node (sinus histiocytosis, lymphoid depletion); adrenals (haemorrhage); thymus (congestion, generalized atrophy, sinus histiocytosis, medullary fibrosis); uterus (luminal dilatation, endometrial stromal polyp) and bone-marrow (femur - depletion of myeloid elements).

Discussion

Pneumonic changes (in different stages) were recorded in good number of cases. The occurrence of these lesions might be due to Mycoplasma sp. which is ubiquitous in nature and is transmitted by intrauterine route or by aerosol between cagemates including dams to offsprings and between adjacent cages (Anon, 1991). It is of common occurrence among conventionally reared rats / mice colonies, however, it rarely complicates toxicological studies (Boorman and Eustis, 1990). Lymphoid hyperplasia in spleen and lymph node in present study might be an immunologic response to pulmonary lesions. Derelanko and Hollinger (1995) also suggested that lymphoid hyperplasia in laboratory rats is a common reactive response to inflammation and systemic infection.

Other lesions in different organs including reproductive organs (refer summary table) were recorded either both in control and treatment groups at comparable level or in a few animals. Moreover these lesions were in accordance with our historical control data, hence considered as spontaneous / incidental findings.

Gross Pathological Findings of Pups

No significant pathological lesion could be detected on external examination of the carcasses.

On post-mortem examination of terminally sacrificed pups belonging to dams of different experimental groups revealed varying degree of lesions in lungs (congestion, haemorrhage, pneumonic changes, emphysema and oedema) and liver (mottling, whitish discolouration, pallor). These changes were recorded either both in control and treatment groups at comparable level or not showed any specific pattern, hence they were considered as a spontaneous / incidental findings.

Comments

No unique lesions were observed in any organs of treated rats that were not detected in concurrent control rats. The background lesions that are normally seen in control rats were similar in treated and the control groups.

The organs that showed significant variations in weight, namely, heart (decrease in treated groups) and liver (an increase in treated groups) did not show any treatment related histopathological lesions. Kidney, the organ for excretion, which potentially has an effect on urinary pH, also did not show any treatment related pathological lesions.

Source : The Dow Chemical Company, Midland, MI.
05.05.2003

(2)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

(1) Unpublished data, The Dow Chemical Company

(2) Unpublished data, The Dow Chemical Company.

10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT

201-16028

4. SUMMARY OF

A. NAME OR C
B. SOLVENT:
C. TEST INIT
NOTE: CONCENT

IESI

NONACTIVATION

SOLVENT CONTROL
POSITIVE CONTRC
TEST COMPOUND

ACTIVATION

PLATE TEST RESULTS

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DMSO
IATION DATE: AUG. 25, 1977
RATIONS ARE GIVEN IN MICROLITE

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LITTON BIONETICS, INC.

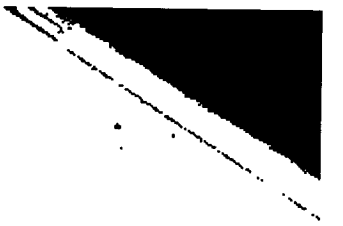
1POUND: RII-35201

RS (UL) OR MICROGRAMS (UG) PER

	BEVERAGINIS		
[A=1535	IA=1537	IA=1538	
1	2	1	2	1	
14		14		16	
000		>1000		>1000	
21		17		25	
26		11		25	
26		20		16	
30		19		20	
23		9		14	

PLATE.

PEB	PLAIE			
	IA=98		IA=100	
2	1	2	1	2	1
	44		89		2
	938		>1000		30
	42		154		1
	22		117		1
	37		142		2
	33		161		1
	30		137		



SOLVENT CONTROL
POSITIVE CONTRO
TEST COMPOUND

*	IBY*	CONVER
**	TA-1535	MN
	TA-1537	QM
	TA-1538	NF
	TA-98	NF
	TA-100	MN
	D4	MN
	SOLVENT	DM

-	RAT	LIVER
)L***	RAT	LIVER
0.001000 UL	RAT	LIVER
0.010000 UL	RAT	LIVER
0.100000 UL	RAT	LIVER
1.000000 UL	RAT	LIVER
5.000000 UL	RAT	LIVER

TANTS PER PLATE

NG	10	UG/PLATE
	10	UG/PLATE
	100	UG/PLATE
	100	UG/PLATE
NG	10	UG/PLATE
NG	10	UG/PLATE
SO	50	UL/PLATE

15	8	27
303	167	904
22	8	21
15	10	24
21	5	13
15	8	19
5	7	9

***	TA-1535	ANTH	100	UG/PL/
	TA-1537	AMQ	100	UG/PL/
	TA-1538	AAF	100	UG/PL/
	TA-98	AAF	100	UG/PL/
	TA-100	ANTH	100	UG/PL/
	D4	DMNA	100	MICRO/
	SOLVENT	DMSO	50	UL/PL/

	49	107
	767	>1000
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	25	105
	36	110
	31	93
	33	5

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